1: Nat Struct Biol. 2001 Mar;8(3):221-5. Related Articles, Links

Comment in:
- Nat Struct Biol. 2001 Mar;8(3):198-200.

Crystal structure of rat biliverdin reductase.

Kikuchi A, Park SY, Miyatake H, Sun D, Sato M, Yoshida T, Shiro Y.

RIKEN Harima Institute/SPring-8, Sayo, Hyogo 679-5148, Japan.
kikuchi@spring8.or.jp

Biliverdin reductase (BVR) is a soluble cytoplasmic enzyme that catalyzes the conversion of biliverdin to bilirubin using NADH or NADPH as electron donor. Bilirubin is a significant biological antioxidant, but it is also neurotoxic and the cause of kernicterus. In this study, we have determined the crystal structure of rat BVR at 1.4 A resolution. The structure contains two domains: an N-terminal domain characteristic of a dinucleotide binding fold (Rossmann fold) and a C-terminal domain that is predominantly an antiparallel six-stranded beta-sheet. Based on this structure, we propose modes of binding for NAD(P)H and biliverdin, and a possible mechanism for the enzyme.

PMID: 11224565 [PubMed - indexed for MEDLINE]

2: Nat Struct Biol. 2001 Mar;8(3):215-20. Related Articles, Links

Comment in:
- Nat Struct Biol. 2001 Mar;8(3):198-200.

Structure of human biliverdin IXbeta reductase, an early fetal bilirubin IXbeta producing enzyme.

Pereira PJ, Macedo-Ribeiro S, Parraga A, Perez-Luque R, Cunningham O, Darcy K, Mantle TJ, Coll M.

Instituto de Biologia Molecular de Barcelona, Consejo Superior de Investigaciones Cientificas, Jordi Girona, 18-26, 08034 Barcelona, Spain.

Biliverdin IXbeta reductase (BVR-B) catalyzes the pyridine nucleotide-dependent production of bilirubin-IXbeta, the major heme catabolite during early fetal development. BVR-B displays a preference for biliverdin isomers without propionates straddling the C10 position, in contrast to biliverdin IXalpha reductase (BVR-A), the major form of BVR in adult human liver. In addition to its tetrapyrrole clearance role in the fetus, BVR-B has flavin and ferric reductase activities in the adult. We have solved the structure of human BVR-B in complex with NADP+ at 1.15 A resolution. Human BVR-B is a monomer displaying an alpha/beta dinucleotide binding fold. The structures of ternary complexes with mesobiliverdin IValpha, biliverdin IXalpha, FMN and lumichrome show that human BVR-B has a single substrate binding site, to which substrates and inhibitors bind primarily through hydrophobic interactions, explaining its broad specificity. The reducible atom of both biliverdin and flavin substrates lies above the reactive C4 of the cofactor, an appropriate position for direct hydride transfer. BVR-B discriminates against the biliverdin IXalpha isomer through steric hindrance at the bilatriene side chain binding pockets. The structure also explains the enzyme's preference for NADP(H) and its B-face stereospecificity.

PMID: 11224564 [PubMed - indexed for MEDLINE]

☐ **3:** Acta Crystallogr D Biol Crystallogr. 2000 Sep;56 (Pt 9):1180-2. Related Articles, Links

Journals Online

Crystallization and preliminary X-ray diffraction analysis of a rat biliverdin reductase.

Sun D, Sato M, Yoshida T, Shimizu H, Miyatake H, Adachi S, Shiro Y, Kikuchi A.

Department of Biochemistry, Yamagata University School of Medicine, Yamagata 990-9585, Japan.

Biliverdin reductase (BVR) catalyzes the final step of haem degradation and converts biliverdin to bilirubin using NAD(P)H as an electron donor. This paper deals with the first crystallization and preliminary crystallographic study of recombinant rat BVR expressed in Escherichia coli. Crystals of BVR were obtained by the sitting-drop vapour-diffusion method. Using synchrotron radiation at station BL44B2 of SPring-8, Japan, BVR diffraction data were collected to 1.6 A resolution. Crystals belong to the orthorhombic space group P2(1)2(1)2(1), with unit-cell parameters a = 58.89, b = 70.41, c = 87.76 A. The complete determination of the crystallographic structure is currently in progress using MAD (multiwavelength anomalous diffraction) data from an Ir-derivative crystal.

PMID: 10957639 [PubMed - indexed for MEDLINE]

☐4: J Biol Chem. 2000 Jun 23;275(25):19009-17. Related Articles, Links

FREE full text article at www.jbc.org

Studies on the specificity of the tetrapyrrole substrate for human biliverdin-IXalpha reductase and biliverdin-IXbeta reductase. Structure-activity relationships define models for both active sites.

Cunningham O, Dunne A, Sabido P, Lightner D, Mantle TJ.

Department of Biochemistry, Trinity College, Dublin 2, Ireland and the Departments of Chemistry & Biochemistry, University of Nevada, Reno, Nevada 89557-0020, USA.

A comparison of the initial rate kinetics for human biliverdin-IXalpha reductase and biliverdin-IXbeta reductase with a series of synthetic biliverdins with propionate side chains "moving" from a bridging position across the central methene bridge (alpha isomers) to a "gamma-configuration" reveals characteristic behavior that allows us to propose distinct models for the two active sites. For human biliverdin-IXalpha reductase, as previously discussed for the rat and ox enzymes, it appears that at least one "bridging propionate" is necessary for optimal binding and catalytic activity, whereas two are preferred. All other configurations studied were substrates for human biliverdin-IXalpha reductase, albeit poor ones. In the case of mesobiliverdin-XIIIalpha, extending the propionate side chains to hexanoate resulted in a significant loss of activity, whereas the butyrate derivative retained high activity. For human biliverdin-IXalpha reductase, we suggest that a pair of positively charged side chains play a key role in optimally binding the IXalpha isomers. In the case of human biliverdin-IXbeta reductase, the enzyme cannot tolerate even one propionate in the bridging position, suggesting that two negatively charged residues on the enzyme surface may preclude productive binding in this case. The flavin reductase activity of biliverdin-IXbeta reductase is potently inhibited by mesobiliverdin-XIIIalpha and protohemin, which is consistent with the hypothesis that the tetrapyrrole and flavin substrate bind at a common site.

PMID: 10858451 [PubMed - indexed for MEDLINE]

☐5: Biochem J. 1997 Nov 15;328 (Pt 1):33-6. Related Articles, Links

Biochem J

Cloning and overexpression of rat kidney biliverdin IX alpha reductase as a fusion protein with glutathione S-transferase: stereochemistry of NADH oxidation and evidence that the presence of the glutathione S-transferase domain does not effect BVR-A activity.

Ennis O, Maytum R, Mantle TJ.

Department of Biochemistry, Trinity College, Dublin, Ireland.

Native biliverdin IX alpha reductase (BVR-A) is a monomer of molecular mass 34 kDa. We have developed an expression vector that allows the isolation of 40 mg of a glutathione S-transferase (GST)-BVR-A fusion protein from 1 litre of culture. The fusion protein (60 kDa) behaves as a dimer on gel filtration (120 kDa), so that we have artificially created a BVR-A dimer. The recombinant rat kidney enzyme exhibits pre-steady-state 'burst' kinetics that show a pH dependence similar to that already described for ox kidney BVR-A. Similar behaviour was obtained in the presence and absence of the GST domain both for the burst kinetics and during initial-rate studies in the presence and absence of albumin. The stereospecificity of the BVR-A-catalysed oxidation of [4-3H]NADH, labelled at the A and B faces, was shown to occur exclusively via the B face.

PMID: 9359830 [PubMed - indexed for MEDLINE]

☐ **6:** Eur J Biochem. 1996 Jan 15;235(1-2):372-81. Related Articles, Links

Human biliverdin IXalpha reductase is a zinc-metalloprotein. Characterization of purified and Escherichia coli expressed enzymes.

Maines MD, Polevoda BV, Huang TJ, McCoubrey WK Jr.

Department of Biophysics, University of Rochester School of Medicine, NY, USA.

Biliverdin IXalpha reductase (BVR) catalyzes the conversion of the heme b degradation product, biliverdin, to bilirubin. BVR is unique among enzymes characterized to date in that it has dual pH/cofactor (NADH, NADPH) specificity. A cDNA clone encoding human BVR was isolated from a gamma library using a probe generated via reverse transcription and the polymerase chain reaction from human placental RNA. This approach was taken because the more direct approach of using the previously isolated rat BVR cDNA as the hybridization probe did not succeed. The human cDNA was cloned and sequenced; it was shown to have an open reading frame encoding a 296-amino-acid protein in which could be identified four peptides previously identified by micro-sequencing purified protein. The cDNA hybridized with a single message of approximately 1.2 kb in human kidney poly(A)-rich RNA, and appeared, by Southern blot analysis, to be the product of a single-copy gene. Sequence analysis indicated that the human reductase shows approximately 83% identity, at both the nucleotide and amino acid levels, with rat BVR. In some regions including the carboxyl terminus, protein sequence identity drops to 45%. Also noteworthy is the presence of two additional cysteine residues in the encoded human reductase (five compared to three for rat). The protein produced by an expression plasmid in which the insert

was cloned in frame with lacZ sequences was characterized, and demonstrated dual pH and cofactor dependence. However, as suggested by kinetic analysis, the human enzyme may also use NADH as cofactor, as opposed to the rat reductase, which most likely utilizes only NADPH under physiological conditions. Western blot analysis and isoelectric focusing demonstrate that, although migrating as a single band on SDS/PAGE, the expressed protein, like that purified from tissue, consists of several isoelectric charge variants. Atomic absorption spectroscopy indicates that the protein purified from human liver contains Zn at an approximately 1:1 molar ratio. That human BVR is a Zn metalloprotein was further substantiated by 65Zn exchange analysis of both the purified and the fusion protein expressed in Escherichia coli. Exogenous Zn also inhibits NADPH-dependent, but not NADH-dependent, activity. Hence, the NADH and NADPH binding regions are differentiated by their ability to interact with Zn; Fe-hematoporphyrin, however, inhibited both NADH- and NADPH-dependent activity.

PMID: 8631357 [PubMed - indexed for MEDLINE]

☐ **7:** Eur J Biochem. 1994 Jun 1;222(2):597-603. Related Articles, Links

Site-directed mutagenesis of cysteine residues in biliverdin reductase. Roles in substrate and cofactor binding.

McCoubrey WK Jr, Maines MD.

University of Rochester School of Medicine, Department of Biophysics, New York 14642.

Biliverdin reductase is unique among all enzymes described to date in having two pH optima, 6.75 and 8.7, at which NADH or NADPH, respectively, are required for activity. The enzyme converts biliverdin to bilirubin in mammals. The mature enzyme, which is 293 amino acids long, has 3 cysteine residues, and is sulfhydryl dependent. To understand the role of the cysteine residues in enzyme activity, we examined the effects of the neutral substitution with alanine of each of three residues, individually and in combination, by site-directed mutagenesis. These residues in the predicted amino acid sequence of rat biliverdin reductase correspond to amino acids 73, 280 and 291. The modification of the amino-proximal cysteine (Cys73), which is flanked by a tyrosine residue, completely inactivated the enzyme with NADH at pH 6.75 and NADPH at pH 8.7. The loss of reductase activity was not due to changes in three-dimensional characteristics of the protein as suggested by its mobility in a non-denaturing gel. Although modification of either of the two cysteines located near the C-terminus (Cys280 and Cys291) significantly reduced activity with both cofactors, these mutations did not inactivate the enzyme. Comparison of Km values for the Cys280-->Ala and Cys291-->Ala mutants with the wild type protein, at pH 8.7, suggests that Cys280 principally functions in substrate binding while Cys291 is predominantly involved in cofactor binding. This assignment probably also applies at pH 6.75. Comparison of kcat of the mutants with wild type shows that

mutation of Cys280 decreases Vmax of the enzyme. Mutation of both C-terminal cysteines caused inactivation of the enzyme, comparable to that produced by mutation of Cys73. Analysis by circular dichroism at far-ultraviolet wavelengths suggests that the alterations in activity are not the result of changes in the secondary structure of these mutants. These results are consistent with Cys73 having a central role in substrate/cofactor binding while biliverdin reductase can function, albeit at a reduced rate, with only one of the near C-terminus cysteines. The results are further consistent with the suggestion that although the two C-terminal cysteines have preferential affinities, they can serve similar functions in the interaction with substrate/cofactor.

PMID: 8020496 [PubMed - indexed for MEDLINE]

☐ **8:** Biochem Biophys Res Commun. 1992 Oct 15;188(1):48-56.

Related Articles, Links

The interplay between basicity, conformation, and enzymatic reduction in biliverdins.

Bari S, Frydman RB, Grosman C, Frydman B.

Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Argentina.

Biliverdins with extended conformations are reduced by biliverdin reductase (BvR) at higher rates than biliverdins with helical conformations. To find out the molecular basis for this important feature of BvR mechanism, helical and extended biliverdins were titrated for their acid-base equilibria in a protic solvent (methanol). It was found that the basicity of biliverdins increases with the stretching of the conformation. Biliverdin IX gamma (all-syn) has a pKa = 3.6; 5,10,15-syn,syn,anti-biliverdin has a pKa = 3.7; 5,10,15-syn,anti,syn-biliverdin has a pKa = 6.1; 5,10,15-syn,anti,anti-biliverdin has a pKa = 6.4; and 5,10,15-all-anti-biliverdin has a pKa = 7.9. The increase in basicity with progressive stretching of conformations closely parallels the increase in the reduction rates by BvR. A biliverdin constrained by a four carbon chain to a helical conformation and which is a very weak base (pKa = 0.4) is not reduced by BvR. Nucleophilic additions of 2-mercaptoethanol at the C10 in biliverdins closely parallel their basicities, as can be expected if the formation of a positive mesomeric species at C10 is linked to the basicity (i.e., the ease of protonation) of the N23 on the pyrrolenine ring.

PMID: 1417867 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text

Items 1-8 of 8 One page.